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SUMMARY OF LABORATORY EXPERIMENTS AND TOXICITY TESTS CONCERNED WITH OIL CONTROL OF PINE BARK BEETLES

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SUMMARY OF LABORATORY EXPERIMENTS AND TOXICITY TESTS CONCERNED WITH OIL CONTROL OF PINE BARK BEETLES

C. B. HENSILL
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INTRODUCTION

Field experiments during 1934, and previous experiments, on the control of bark beetle broods by oils, have shown variable results and inconsistencies under apparently identical conditions. The isolation of any one factor or combination of factors, such as bark density, thickness and texture (resistance to oil penetration) in relation to brood mortality failed to reveal any appreciable correlation when equal applications of oil were used.

It was considered that this variation in results might be due to (1.) resistance of the insects to action of the oils because of some obscure condition of metabolism or stage of development, or (2) failure of the oils to reach the broods through the bark due to variation in bark characteristics.

In order to eliminate any factors attributable to the bark which might affect the results, it was planned to test various brood stages in the laboratory after the insects had been removed from the bark. This was done by exposing the broods to the oils by a method which insured absolutely uniform conditions of exposure. Such a procedure was followed, first, to determine if the broods displayed resistance to the oils and to what extent. The second point to be considered in a like manner was the testing of materials other than those already used in order to obtain higher toxicity to the insects and better penetration of the bark. It was also decided that tests of some of the formulae would be made to determine whether the fumigation or contact effect of the oil mixture was most important.

From the factors considered above the four series of experiments to be presented in the body of this report were outlined. These experiments were conducted at the Berkeley laboratory during the period of November, 1934, to April, 1935.

I. Series A.

Objective: To determine the existence and extent of resistance of D. b. broods to oil formula No. 21046 of the Standard Oil Co.

Methods: 1. These tests were made on mature larvae of D. b. by a contact exposure method of immersing the larvae in the oil solution.

2. Larvae were used in unit groups of twenty-five for each period of exposure.

3. Groups of larvae were submitted to the following periods of exposure to the oils: one second, fifteen seconds, thirty seconds and one, two, four and eight minutes. While being immersed, the larvae were held in a small basket made of fine-mesh wire screen. (Pl.1.) At the end of the period of immersion each group of larvae was removed from the screen basket and allowed to drain on an oil-absorbing material, masonite being used in this case. They were then placed in bark cells for observation. (Pl.2.) and kept at a temperature of 70- 75° Fahr.

4. Mortality counts were made at regular intervals beginning twenty-four hours after treatment.(Pl.3)

Results: 1. The results of representative tests are presented in Table 1 and figure 1 (See Appendix).

TABLE I.

Resistance of D.b. brood to Oil treatment with Standard Oil Co. formula No. 21066-R

Brood Stage	Per Cent Mortality in Intervals after Treatment											
	Mature Larvae				Medium Larvae				Small Larvae			
Exposure Period	1 Sec.	1 Min.	4 Min.	8 Min.	1 Sec.	1 Min.	4 Min.	8 Min.	1 Sec.	1 Min.	4 Min.	8 Min.
1 DAY	4	16	32	68		16	44	60	8	32	76	80
2 "	16	32	64	84	8	28	68	72	28	84	88	84
3 "	24	44	72	96					32	64	88	88
4 "	24	52	84	96	20	44	88	100	40	76	96	94
5 "	28	60	88	96					52	84	96	94
6 "	44	72	92	100	32	52	88		52	84	96	98
7 "	48	76	92						60	84	96	100
8 "	48	76	92		40	60	88		64	84	96	
10 "	56	80	96		52	56	90		76	84	100	
12 "	60	92	100		72	66	96		84	92		
14 "	80	92			88	84	100		88	96		
16 "	88	100			92	96			96	100		
18 "	88				96	100			100			
20 "	92				100							
22 "	96	(Mortality of checks was 24% in 24 days for large larvae; 44%										
24 "	100	in 20 days for medium larvae, and 76% in 18 dys. for small lar.)										
	NEW ADULTS											
1 "	4	32	60	80								
2 "	36	60	80	92								
3 "	72	72	92	96								
4 "	76	92	100	100								
5 "	80	96										
8 "	96	100										
10 "	100											
(Mortality for check was 24% in 4 dys.)												
	PUPAE											
1 DAY	1 Sec.	10 Sec.	30 Sec.	40 Sec.	80 Sec.							
	100	100	100	100	100							
(10% mortality in check in 1 days.)												

2. It is evident from the data presented that the larvae of D.b. do exhibit resistance to the oil. Some are so resistant as to live for several days after drastic treatment.

3. That different larvae vary in the extent of resistance is evident from the data on small larvae in Table I., where the mortality was higher in part at four minutes exposure to the oil than it was at eight minutes. Other similar cases were also evident throughout the experiments.

4. Apparently the most resistant brood stage of D. b. is the mature larvae followed in order by the medium larvae, small larvae, adults and pupae.

5. As a rule the rate of mortality of D. b. brood varies directly with the period of exposure to the oil.

6. No larvae developed into pupae after any of the above treatments. All eventually succumbed though much resistance was shown in some cases. Most of the larvae in checks developed to adults.

7. The variation in resistance exhibited by the larvae helps to explain the lack of correlation which has been obtained in field work, as different larvae do vary in their resistance.

II. Series B.

Objective: To test the toxicity of various oil formula against bark beetle larvae.

Methods: The methods employed in this series of experiments were the same as those employed in series A.

Results: 1. Results of some representative tests from this series are presented in Table II and in figure 2 (See Appendix).

T A B L E II

Toxic effects of various Oil Mixtures on Mature Larvae of D. brevicornis

OIL FORMULA	% Mortality in 24 Hours				% Mortality In 5 Days				% Mortality in 10 Days			
Exposure Period	1 Sec	1 Min	4 Min	8 Min	1 Sec	1 Min	4 Min	8 Min	1 Sec	1 Min	4 Min	8 Min
1. Oil 24241-R 90% & " 24240-R 10% with naphthalene 1# per gal. and beta naphthol 1/3 " "	28	36	68	76	80	72	100	100	100			
2. Stainless, penetrating creosote	0	30	100	100	40	50			80	100		
3. Standard Oil Co. formula No. S1046-R	4	16	32	68	28	60	88	96	68	80	96	100
4. Same as 1. with beta naphthol 1/3 lb. per gallon	16	74	98	100	52	98	100	44	100			
5. Black creosote.	10	32	35	45	65	85	95	90	95	100	100	100
6. Black creosote 50% and oil 24241-R 50%	30	50	70	80	70	80	80	90				
7. (24241-R 90% and 24240 10%) ½ with stainless creosote ½ and beta naphthol 1/3 lb. per gallon.		65		86		90				100		
8. Organic solvent 1/3 and heavy lubricating oil 2/3 with beta naphthol 11/3 lb. per gallon		60				100						
9. Organic solvent 25% and heavy lubricating oil 75% with naphthalene 50 gm. per 100 c.c.		80				95				100		
10. (Oil 24241-R 90% and oil 24240-R 10%) ½ with heavy lubricating oil ½ and beta naphthol 1/3 lb. per gallon				68								
11. (Oil 24241-R 50% and oil 24240-R 50%) with naphthalene 1/2 lb per gal.	8	17	16	4	16	48	64	48	80	54	80	64
12. (Oil 24241-R 75% and oil 24240-R 25%) with naphthalene 1/2 lb. per gal.	0	0	0	4	20	11	12	16	33	28	25	44
13. (Oil 24241-R 25% and oil 24240-R 75% with paradichlorobenzene 1/3 lb. per gallon	4	8	16	12	20	36	48	36	48	72	80	72
14. Chlorinated naphthalene (medium sized larvae)	4	4	16	16	44	28	44	44	76	64	64	72

Average mortality of checks was approximately 25%.

NOTE: Oils 24241-R and 24240-R are base oils provided by the Standard Oil Co.

2. Formula number 21046 of the Standard Oil Company continued to give good results with consistently high toxicity.

3. Certain other formulae gave very good results in these tests and it is considered that further experimentation with them is worthwhile. These are formulae 1, 2, 5, 6 and 7.

4. No development of treated larvae took place when they were treated with the formulae mentioned in the two previous paragraphs.

5. Formulae 8 and 9, in which organic solvents were employed in order to put more of the chemicals into solution, also were high in toxicity but would be very costly.

6. All materials tested were toxic to the larvae but some were not sufficiently so. Some of the oils alone without chemicals in solution were toxic but very slow in their action.

7. Tests of formulae 11, 12 and 13 which were used in experimental control work in the fall of 1934 showed them to be slow in action and variable in toxicity, as was found in the field experiments.

8. From the standpoint of contact toxicity, much promise is shown by beta naphthol which had not been worked with previous to these tests.

III. Series C.

Objective: Fumigation tests of oil formulae.

Methods: 1. These tests were conducted by sealing the beetles in unit groups in vials with the materials being tested. A screen of fine mesh placed in the vial prevented them from contacting the material.

2. Where chemicals alone were tested, the beetles were sealed in the same vial with the chemical (Pl.4).

3. Where the ability of the oil to carry fumes of the chemical through bark or masonite was being tested, setups as shown in plate 5 were used.

4. Adults were used in these tests due to the fact that it is less difficult to determine mortality due to fumigation in the case of adults than in the case of larvae or pupae.

5..Periods of exposure of the beetles to fumigation varied according to the tests being conducted.

6. Mortality counts were made as in the previous series of tests.

Results: 1. Data from representative tests of this series is presented in Tables III and IV.

TABLE III

Tests of Fumigative Effects of Chemicals on P. h. Adults after Twenty-five Hours Exposure

No. Adults	Chemical	PER CENT MORTALITY Days after Treatment 2	3	6	7	9
20	Naphthalene	70	85	100		
20	Beta naphthol	65	85	95	100	
20	Paradichlorobenzene	25	35	70	95	100
20	Benzene hexachloride	30	50	100		
20	Check	10	25	65	95	100

T A B L E IV

Conduction of Chemicals through Bark of Ponderosa Pine by Oils

Oil Formula	Adults:	Exposure: Period	No Days - 1	2	3	4	5	6	7	8	9	Per Cent Mortality 10
24241-25%;24240-75%: with paradichloro-benzene	20	90 hrs:	20:	35:	45:	65:	80:	95:	:	:	:	
Same oil formula without paradichlo-robenzene	20	90"	10:	25:	45:	40:	55:	65:	:	:	:	
Check	15	:	0:	0:	30:	30:	60:	60:	2	:	:	
Oil 24241- 50% and 24240-50% with naph-thalene 1/2 lb.per gallon	25	48 "	24:	44:	44:	72:	80:	84:	92:	92:	96:	100
Same Oil formula without naphthalene:	25:	48 "	0:	12:	12:	56:	68:	76:	76:	76:	88:	92
Check	25:	:	0:	8:	8:	40:	64:	80:	84:	84:	96:	96
24241-90% and 24240: 10%, with beta naph-thol 1/3 lb. per gal	40:	48 "	25:	45:	58:	58:	75:	75:	77:	98:	98:	98
Same oil formula with naphthalene 1/2 lb. per gallon	40:	48 "	75:	88:	95:	98:	95:	95:	100:	:	:	
Same oil formula without chemical	40:	48 "	20:	45:	75:	75:	93:	93:	98:	98:	98:	98
Check	40:	:	17:	45:	68:	68:	82:	82:	93:	93:	93:	98
Standard Oil Co. Formula 21046-R (thru masonite)	10:	100 "	:	80:	90:	100:	:	:	:	:	:	

2. Of the four chemicals tested directly (without being dissolved in oil (Table III)) as to their efficacy as fumigants, the following order of decreasing toxicity is apparent: naphthalene, benzene hexachloride, beta naphthol and paradichlorobenzene. It is to be noted that this order is not the same when the materials are dissolved in oil (Table IV).

5. The fumes of naphthalene, paradichlorobenzene and beta naphthol are carried through porous substances, such as bark and masonite, by the oils. It is evident from the data in Table IV, however, that the ease with which this is done is variable. Naphthalene appears to be the strongest fumigant of the three and most readily carried through porous materials by the oils.

4. Toxicity due to oils is apparently due to contact, as there appears to be very little fumigative effect without a chemical being dissolved in the oil

4. Series D. Miscellaneous Experiments.

A. Experiments with D. monticolae.

Objective: To test the resistance of various brood stages of Dendroctonus monticolae to Standard Oil Co. formula No. #1046-B.

Methods: 1. The methods employed in these tests were the same as those employed in Series A and B.

Results: 1. Results of these experiments are presented in Table V.

TABLE V

Resistance of D.M. brood to Standard Oil Co. Formula #21046-B

Period of Exposure to Oils	Mortality in 24 Hours
Mature Larvae	
1 second	98
1 minute	88
4 "	100
8 "	100
Check	0
Medium Larvae	
1 second	96
1 minute	100
4 "	100
8 "	100
Check	8
Adults	
1 second	75
1 minute	100
4 "	83
8 "	92
Check	88

2. It is apparent that the brood of D. m. is much less resistant to oil treatment than the brood of D. b. Though the pupae were not tested, it is most likely that they are even more easily killed than those of D. b.

3. Very little indication of the difference in resistance of the brood of these two species has been noted as yet in field work. This is no doubt due to the differences in bark characteristics of ponderosa pine and sugar pine.

B. Oil Penetration Tests.

Objective: To test the rate of penetrativity of various oils through masonite and ponderosa pine bark.

1. Tests with Masonite.

Methods: 1. Strips of this material 4 in. long and 1/2 in. square were used with same set-up as recorded in the report of Struble and Hensill.*

2. The time required for the oil to rise along the length of each strip was recorded.

Results: 1. The following results were obtained from the average of two strips for each oil:

Oil Formula	Time required to rise length of strip
Stand. Oil Co. formula #21046-H	56 min. 30 sec.
#24241-90% and #24240- 10%	29 " 45 "
#24241-75% and #24240- 25%	37 " 30 "

2. Tests with ponderosa pine bark.

Methods: 1. Strips of bark 2-1/4 in long by 1 in. wide by 1/4 in. thick were treated as above.

Results: 1. The following results were obtained:

Oil Formula	Time required to rise length of strip
Stainless, penetrating creosote	11 min.
Cabinol (steam distilled pine oil)	32 "
Oil 24241,90% and Oil 24240,10%	19 "

*See References

3. It is to be kept in mind with regard to the two tests above that the masonite is a material which is uniform in texture, while bark is usually quite variable.

4. The variation of oils in their ability to penetrate porous materials is evident from the data presented.

C. Brood Mortality tests in bark.

Objective: To test the efficiency of various oil formulae when applied to brood of D.b.in the brood bark.

Methods: 1. Sections of brood bark of approximately one square foot area were used.

2. Oils were applied by pouring a light coat on the inner surface and a heavy coat on the outer surface. After treatment the bark sections were kept out of doors in the shade at a mean temperature of about 80° F.

3. Mortality counts were made by shaving up the bark and tabulating dead and living larvae.

Results: 1. Results of these tests are presented in Table VI.

T A B L E VI

Mortality of D.h. larvae in Brood Bark Treated with various oil Formulae

Oil Formula	Days Exposure	Brood Mortality
1. Standard Oil Co. No.21040	20	95.6%
2. Oil 24241, 90% and 24240,10%: plus beta naphthol 1/3 lb.per gal	20	76.7%
3. Oil 24241,75% and 24240, 25%: with beta naphthol same amount	20	97.2%
4. Oil 24241,90% and 24240, 10%: 1/2 with stainless creosote 1/2: and beta naphthol 1/6 lb.per gal:	20	90. %
5. Oil 24241,75% and heavy lubricating oil 25% with beta naphthol 1/3 lb. per gallon	19	94. %
6. Organic solvent 25% and heavy lubricating oil 75% with naphthalene 1 lb. per gallon	19	100. %
7. Oil 24241, 90% and 24240,10%: with naphthalene 1 lb. per gal. and beta naphthol 3/4 lb. per gallon.	13	80. %
Check		0.0%

Bark thickness averaged 1-1/2 inches

2. There is some lack of correlation between the bark tests above and the toxicity tests previously presented. This is most likely due to variation in oil penetration. It shows the advantage of tests of this type as well as field tests of a similar nature, in order to determine the proper oil viscosity to be used.

CONCLUSIONS

1. The various brood stages, of the D.b. in particular as well as D.m., were found to have varying resistance to formula No. E1046-R of the Standard Oil Co., a naphthalene mixture. The order of resistance for both species was large larvae the most resistant, then medium larvae, small larvae, and adults and pupae. The brood of D.m. is less resistant than D.b. Very few cases of development of oil treated larvae were observed while the checks continued to develop with an average loss of approximately 25%.

2. Some new toxic materials and mixtures were developed. some of which show considerable promise for future work.

3. Naphthalene was found to be the strongest chemical used as a fumigant and beta naphthol the strongest as a contact poison. The combination of these materials was found to be very advantageous.

4. It was found that the fumes of the various chemicals dissolved in the oils were carried through the bark by the oils. Some, however, were carried more readily than others.

5. The rate of penetration of various oil formulae in bark and masonite was found to vary with the viscosity of the oils. This point is of importance in field practice as the oil must not be too thin or too thick, in order that optimum penetration be obtained.

6. The above point was brought out in some mortality tests on brood in the bark with various oil formula, where it was found that mixtures which were toxic by a contact exposure method of testing were not as toxic in this manner due to inefficient penetration.

7. The important points for continued field work on this problem are considered to be the further development of the formulae in Table VI, especially as regards the proper oil viscosity. Any improvements in technique of application along with this development will also be of advantage.

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Plate 1.
Screen basket used for immersing groups of larvae in oils.
(Natural size)

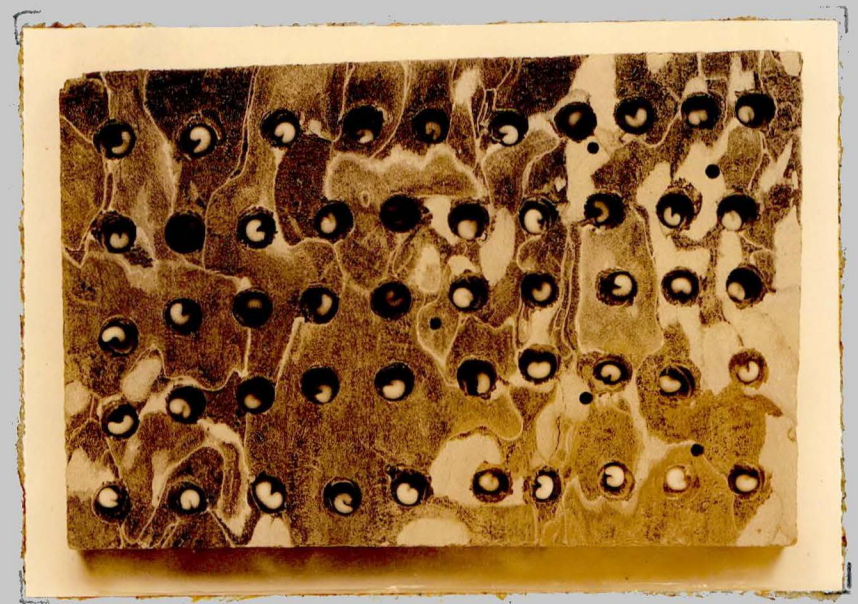


Plate 2.
Bark cells used as containers for larvae after oil treatment. (Natural size.) This was covered by a thin flat piece of bark of the same size- the two being held together with rubber bands.

Plate 3.

Left- Normal, mature D. b. larvae.
Right- Typical appearance of same larvae killed by oil. Black specks caused by sawdust.
(Twice natural size.)

Plate 4.

Apparatus used for testing fumigative strength of various chemicals against adults of D.b. Beetles were held in wire basket above the chemical.
(One-half natural size.)

-A- -B-

-C-

Plate 5. Set-ups used for determining the ability of oils to carry fumes of chemicals through bark and masonite.
A. Section of bark before inserting vial
B. Complete set-up with vial waxed onto bark and oil all absorbed by bark.
C. Set-up used to determine penetration through masonite strips.
(1/2 natural size.)

FIGURE I. RESISTANCE OF D.B. BROOD TO OIL TREATMENT WITH STANDARD OIL CO. FORMULA NO. 21046-R AT VARIOUS PERIODS OF EXPOSURE.

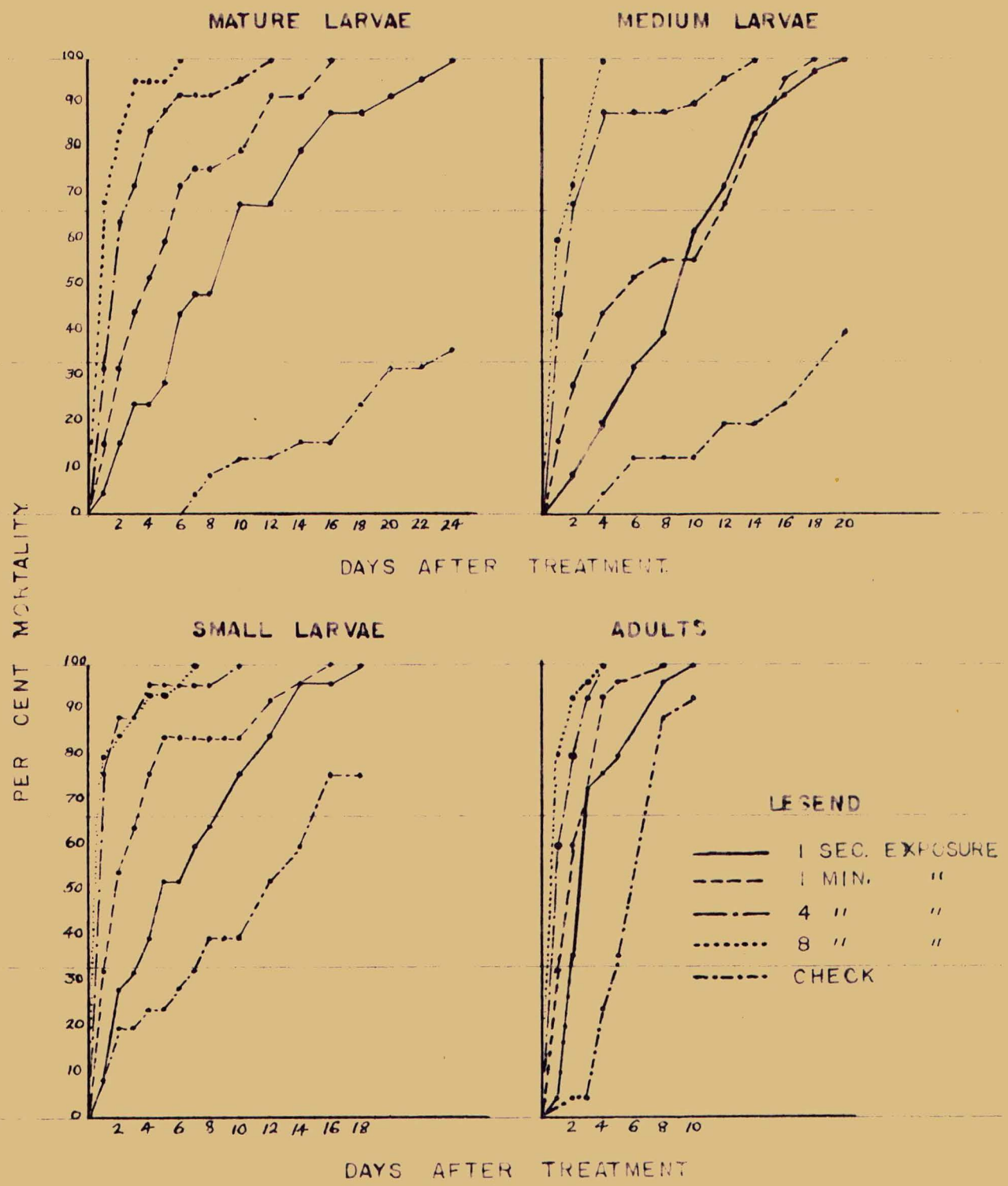
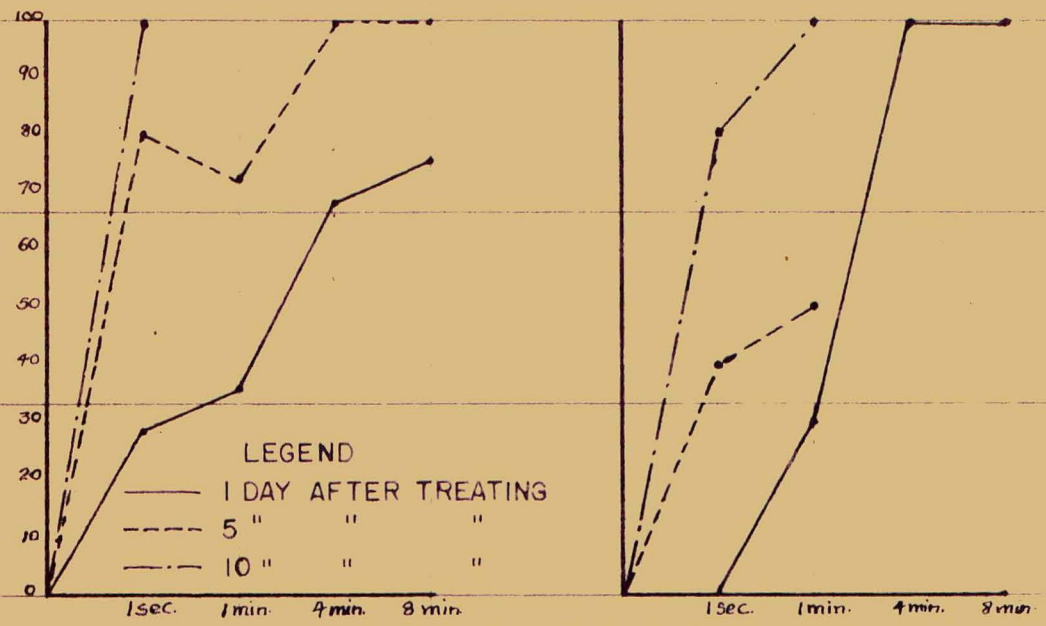


FIGURE 2. TOXICITY OF OIL MIXTURES TO MATURE D.B. LARVAE.

OIL 24241-R 90% OIL 24240-R
10% WITH NAPHTHALENE 1 LB.
AND BETA NAPHTHOL 3/4 "
PER GALLON.

STAINLESS, PENETRATING CREOSOTE.



PER CENT MORTALITY.

PERIOD OF EXPOSURE

STANDARD OIL CO. FORMULA NO. 21046-R.

BLACK CREOSOTE.

PERIOD OF EXPOSURE.